[ψ13-14] Bombesin Analogues Inhibit Growth of Small Cell Lung Cancer in Vitro and in Vivo

Samira Mahmoud, Julie Staley, John Taylor, Arthur Bogden, Jacques-Pierre Moreau, David Coy, Ingalill Avis, Frank Cuttitta, James L. Muslinhe, and Terry W. Moody

ABSTRACT

Bombesin/gastrin releasing peptide (BN/GRP) functions as an autocrine growth factor in small cell lung cancer (SCLC). Previously, this autocrine growth cycle was disrupted by a monoclonal antibody which binds to the carboxy-terminal of BN and neutralizes the peptide so that it is unable to interact with the BN/GRP receptor. Here a series of BN analogues were synthesized which have a reduced peptide bond near the carboxy-terminal. The analogues inhibited specific binding of 125I-GRP to SCLC cell line NCI-H345 in a dose-dependent manner and the analogue [ψ-Arg1, D-Pro2, D-Trp7-9, Leu16]BN6-14 was approximately 6-fold more potent than BN. BN6-14 with a 50% inhibition concentration of 5 nM, [ψ-Arg1, ψ-Arg1, D-Pro2, D-Trp7-9, Leu16]BN6-14 and [ψ-Arg1, Leu16]BN6-14 had no effect on the cytosolic Ca2+ levels but antagonized the increase in cytosolic Ca2+ caused by 10 nM BN. [ψ-Arg1, ψ-Arg1, Leu16]BN6-14 and [ψ-Arg1, Leu16]BN6-14 respectively inhibited the growth of SCLC in vitro using a clonogenic assay by approximately 50% and 70% respectively. Inclusion of [ψ-Arg1, Leu16]BN6-14 (10 µg, s.c.) inhibited the growth of SCLC xenografts in nude mice in vivo by approximately 50%. These data suggest that the autocrine growth cycle of BN/GRP in SCLC may also be disrupted by peptide antagonists which bind to the BN receptor.

INTRODUCTION

Bombesin/gastrin releasing peptide is a pulmonary growth factor which fulfills criteria for autocrine stimulation. Most SCLC cell lines and tumors produce BN/GRP (1-4). BN/GRP is derived from high molecular weight precursor proteins whose mRNA has been detected in tumors and cell lines (5, 6). After posttranslation processing, BN/GRP is secreted from SCLC cells into the tissue culture media (7, 8). In addition, elevated plasma levels of BN/GRP are present in SCLC patients with extensive but not limited disease (8-10). When secreted BN-like peptides interact with SCLC cell surface receptors (11). BN or GRP bind with high affinity (Kd = 1 nM) to SCLC protein receptors (12). BN stimulates phosphatidylinositol turnover in SCLC cells (13) and the resulting metabolite inositol-1,4,5-trisphosphate causes release of Ca2+ from intracellular stores (14, 15). Also, BN or GRP stimulate the growth of SCLC cells in a clonogenic assay (16) and the growth of SCLC xenografts in nude mice (17).

The growth of SCLC in vitro and in nude mice in vivo is greatly inhibited by a monoclonal antibody (2A11) against BN (18). Subsequently, a phase I clinical trial was conducted which indicated that the effects of BN/GRP can be partially blocked by 2A11 without apparent clinical toxicity to the patient (19). An alternative way to disrupt the BN-induced autocrine growth cycle of SCLC is to use BN/GRP receptor antagonists. Such analogues may also be disrupted by peptide antagonists which bind to the BN receptor.

MATERIALS AND METHODS

Cell Lines. The continuous clonal cell line NCI-H345 was grown as a suspension culture in SIT medium supplemented with 2.5% fetal bovine serum and cell lines NCI-H520, H69, H720, H727, and N592 were grown in serum supplemented (RPMI 1640 containing 10% fetal bovine serum) medium (24). Selenium, insulin, and transferrin were purchased from Sigma Chemical Co., St. Louis, MO, and fetal bovine serum was purchased from Gibco.

Receptor Binding Studies. Cell lines NCI-H345 or H720, which have approximately 1500 BN/GRP receptors/cell were harvested 1 day after a medium change; cell lines NCI-H69 and N592 only weakly bound 125I-GRP, whereas cell lines NCI-H727 (lung carcinoid) and NCI-H520 (squamous cell carcinoma) did not specifically bind 125I-GRP. NCI-H345 or H720 cells (2 x 10⁵) were incubated with 0.25 nm 125I-GRP (Amersham Corp., Arlington Heights, IL) at 25°C for 30 min in medium (SIT containing 0.25% bovine serum albumin (Miles Laboratories, Naperville, IL) and 100 µg/ml bacitracin (Sigma Chemical Co.) in the presence or absence of unlabeled peptide. Bound 125I-GRP was separated from free by using the centrifugation techniques described previously. The reduced peptide bond BN analogues were synthesized by using the solid phase methodology described recently (25).

Cytosolic Ca2+ Levels. Cell lines NCI-H345 or H720 were harvested and the cells (2.5 x 10⁶/ml) incubated with Fura 2 AM at 37°C for 30 min (24). The cells which contained loaded Fura 2 were centrifuged at 150 x g for 10 min and resuspended at the same concentration in new SIT medium. The fluorescence intensity was continuously monitored by using a spectrofluorometer equipped with a magnetic stirring mechanism and temperature (37°C) regulated cuvet holder prior to and after the addition of peptide as described previously (14). SCLC cell lines NCI-H69 and N592, only weakly elevated the cytosolic Ca2+.

Clonogenic Assay. Cell lines NCI-N592, H69, H520, H720, H727, and H345 were harvested and tested in the agarose cloning system described previously (22). The base layer consisted of 3 ml of 0.5% agarose in SIT medium containing 5% fetal bovine serum in 6-well
plates (Falcon, Oxnard, CA). The top layer consisted of 3 ml of SIT medium in 0.3% agarose, the peptide(s) doubly concentrated, and 6 × 10^4 single viable cells. For each cell line and peptide concentration, triplicate wells were plated. After 2 weeks, 1 ml of 0.1% p-iodonitrotetrazolium violet was added and after 16 h at 37°C the plates were screened for colony formation; the number of colonies larger than 120 μm in diameter were counted, using an Omnicon image analysis system (Bausch & Lomb, Rochester, NY).

Growth Studies in Vivo. Female athymic BALB/c nude mice, 4–5 weeks old, were housed in a pathogen-free temperature controlled isolation room and were exposed to a light regimen of 7:00 a.m. to 7:00 p.m. The diet consisted of autoclaved rodent chow and autoclaved water given ad libitum. NCI-N592 or H69 cells (1 × 10^7) were injected into the right flank of each mouse by s.c. injection (18). Palpable tumors were observed in approximately 80% of the mice after 7 days. Palpable tumors did not form 3 weeks after injection of NCI-H345 or H720. The tumor-bearing mice were given injections daily of 100 μl of PBS (there was no difference in xenograft formation for control mice which received no injection, PBS, or SIT medium) or PBS containing 10 μg of peptide into the s.c. tissue adjacent to the tumor nodule. The tumor volume (height x width x depth) was determined weekly by calipers and recorded. After 5 weeks, the mice were sacrificed and the tumors were excised, weighed, and frozen in liquid nitrogen. DNA content was determined by using a modification of the phenylamine procedure with calf thymus DNA as a standard (26). The protein content was determined by using the method of Lowry et al. with bovine serum albumin as standard (27).

RESULTS

Receptor Studies. Previously, we found that 125I-GRP bound with high affinity (Kd = 1 nM) to a single class of sites (1500/cell) using SCLC cell line NCI-H345 (12). Here [d-Nal^6, Phe^13-14]BN^6-14 inhibited specific 125I-GRP binding to SCLC cells in a dose-dependent manner. Fig. 1 shows that specific 125I-GRP binding was not inhibited by 0.5 nM [d-Nal^6, Phe^13-14]BN^6-14 whereas all specific specific binding was inhibited by 1 μM [d-Nal^6, Phe^13-14]BN^6-14. The concentration of [d-Nal^6, Phe^13,14]BN^6-14 that inhibited 50% of the specific 125I-GRP binding was 5 nM; the IC50 for BN was 1 nM. Also, [d-Nal^6, Phe^13-14]BN^6-14 and [Phe^6, Phe^13,14, Leu^14]BN^6-14 and [Leu^13,14, Leu^14]BN^6-14 inhibited specific 125I-GRP binding to NCI-H345 in a dose-dependent manner and the IC50 values were 15, 25, and 30 μM, respectively (Table 1). Similar data were obtained with NCI-H720 (data not shown).

Signal Transduction. When SCLC cell line NCI-H345 is loaded with the fluorescent Ca2+ indicator Fura 2 AM, 10 nM BN or GRP increased the cytosolic Ca2+ levels from 140 ± 10 to 180 ± 10 nM. Fig. 2 shows that 1 or 10 nM [d-Nal^6, Phe^13,14, Phe^14]BN^6-14 had no effect on the cytosolic Ca2+ levels. When 10 nM BN was subsequently added there was a strong increase in the cytosolic Ca2+ levels (Fig. 2, A and B). Similarly 100 and 1000 nM [d-Nal^6, Phe^13,14, Phe^14]BN^6-14 had no effect on the basal Ca2+ levels (Fig. 2, C and D), and when 10 nM BN was subsequently added, the Ca2+ response was weak and abolished, respectively. These data indicate that [d-Nal^6, Phe^13,14, Phe^14]BN^6-14 inhibits the BN-induced increase in cytosolic Ca2+ in a dose-dependent manner.

Table 1 Inhibition of binding by BN/GRP receptor antagonists

<table>
<thead>
<tr>
<th>Peptide</th>
<th>IC50 (nM)</th>
</tr>
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<tbody>
<tr>
<td>[d-Nal^6, Phe^13,14]BN^6-14</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>[d-Nal^6, Phe^13,14, Leu^14]BN^6-14</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>[Phe^6, Phe^13,14, Leu^14]BN^6-14</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>[Phe^13,14, Leu^14]BN^6-14</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>[Pyr^6, Phe^13,14, Leu^14]BN^6-14</td>
<td>50 ± 12</td>
</tr>
<tr>
<td>(APTTL)SPBN^1-2</td>
<td>&gt;10,000</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of [d-Nal^6, Phe^13,14, Phe^14]BN^6-14 ([N Ps1 P]BN^6-14) on cytosolic Ca2+. The ability of (A) 1 nM (N Ps1 P)BN^6-14, (B) 10 nM (N Ps1 P)BN^6-14, (C) 100 nm (N Ps1 P)BN^6-14, (D) 1000 nm (N Ps1 P)BN^6-14, and (E) 1 μM BN^1-12 to alter the cytosolic Ca2+ was determined before and after administration of 10 nM BN.
Table 2 Effect of BN-like peptides on NCI-H345 cytosolic Ca\(^{2+}\) and clonogenic growth

Peptides were added to cell line NCI-H345 at a 1 µM dose and the ability to inhibit cytosolic Ca\(^{2+}\) and growth stimulated by 10 nM BN was determined; —, inhibition of response; —, inactive. Similar data were obtained with NCI-H720.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Cytosolic Ca(^{2+})</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>[D-Nal(^6), Psi(^{13-14}), Phe(^{14})]BN(^{6-14})</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[D-Nal(^6), Psi(^{13-14}), Leu(^{14})]BN(^{6-14})</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[D-Phe(^6), Psi(^{13-14}), Leu(^{14})]BN(^{6-14})</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[Psi(^{13-14}), Leu(^{14})]BN</td>
<td>—</td>
<td>ND*</td>
</tr>
<tr>
<td>[Pyr(^{6}), Phe(^{13}), Psi(^{13-14}), Leu(^{14})]BN(^{6-14})</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(APTTL)SP</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

* ND, not determined.

Fig. 3. Reversibility of (N Psi P)BN\(^{6-14}\). The effect of 1 µM (N Psi P)BN\(^{6-14}\) on the cytosolic Ca\(^{2+}\) was determined prior to the addition of 1, 10, and 1000 nM BN.

Fig. 4. Effect of [Psi\(^{13-14}\), Leu\(^{14}\)]BN on colony formation. The number of colonies using SCLC cell line NCI-H345 and NCI-N592 was determined in the absence (column C) and presence of 10 nM BN (column B); 50 nM [Psi\(^{13-14}\), Leu\(^{14}\)]BN (column A); and 10 nM BN + 50 nM [Psi\(^{13-14}\), Leu\(^{14}\)]BN (column C + A). Columns, mean value of 3 determinations is indicated (P < 0.05, *); bars, SE.

growth Studies In Vitro. In a clonogenic assay, BN stimulates the growth of SCLC cell lines. Fig. 4 shows that 10 nM BN stimulated significantly the growth of SCLC cell lines NCI-H345 and NCI-N592 (210 and 160%, respectively). With cell line NCI-H345, 50 nM [Psi\(^{13-14}\), Leu\(^{14}\)]BN decreased significantly the number of colonies from 403 to 97 and from 646 to 249, using cell line NCI-N592. In the presence of 10 nM BN, 50 nM [Psi\(^{13-14}\), Leu\(^{14}\)]BN decreased the colony count from 855 to 424, using cell line NCI-H345, and from 1029 to 632, using cell line NCI-N592. Therefore [Psi\(^{13-14}\), Leu\(^{14}\)]BN decreased SCLC colony formation in the absence or presence of exogenous BN. Similarly, 1 µM [D-Nal\(^6\), Psi\(^{13-14}\), Leu\(^{14}\)]BN\(^{6-14}\), [D-Nal\(^6\), Psi\(^{13-14}\), Phe\(^{14}\)]BN\(^{6-14}\) and (APTTL)SP inhibited colony formation of classic SCLC line NCI-H345 (Table 2). In contrast, BN\(^{11-12}\), which is inactive, had no effect on the growth of cell lines NCI-H345, N592, and H720 (Table 3). Also, Table 3 shows that 1 µM [Psi\(^{13-14}\), Leu\(^{14}\)]BN inhibited most of the colony formation of NCI-H345, N592, and H720, whereas it had no effect on control lines NCI-H520 and H727 which have EGF but not BN/GRP receptors.

Growth Studies In Vivo. The ability of [Psi\(^{13-14}\), Leu\(^{14}\)]BN to antagonize the growth of SCLC in nude mice was investigated.

Table 3 Effect of BN analogues on the growth of lung cancer cell lines in vitro

The mean colony count of 3 determinations in the presence or absence of BN-like peptide was determined. The mean value ± SE is indicated.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>1 µM</th>
<th>1 µM BN(^{11-12})</th>
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<tbody>
<tr>
<td>NCI-H345</td>
<td>39 ± 4*</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>NCI-H520</td>
<td>90 ± 10</td>
<td>ND*</td>
</tr>
<tr>
<td>NCI-H592</td>
<td>24 ± 2*</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>NCI-H720</td>
<td>33 ± 4*</td>
<td>110 ± 6</td>
</tr>
<tr>
<td>NCI-H727</td>
<td>105 ± 11</td>
<td>ND</td>
</tr>
</tbody>
</table>

* P < 0.05.

* ND, not determined.

Fig. 5. Effect of [Psi\(^{13-14}\), Leu\(^{14}\)]BN on xenograft formation in nude mice. NCI-N592 cells were injected s.c. into nude mice and after 1 week the average tumor volume was determined weekly. (A) Five mice were given injections of s.c. daily of PBS (O) or PBS containing 10 µg of [Psi\(^{13-14}\), Leu\(^{14}\)]BN (•) and 10 µg of (APTTL)SP (A) s.c. adjacent to the tumor. Points, mean of 3 determinations is indicated; bars, SE. (B) 3 mice received PBS (O) daily for weeks 1-5 or PBS containing 10 µg of [Psi\(^{13-14}\), Leu\(^{14}\)]BN (•) and 10 µg of BN\(^{11-12}\) (•) (P < 0.05; •).
The mean value ± S.E. of 3 tumors is indicated.

One week after injection of NCI-N592 cells, a palpable mass formed (Fig. 5). In the first study, the growth of tumors in which the mice were given injections of PBS increased exponentially and the tumor volume increased during weeks 1 to 5 from 8 to 1700 mm³ (Fig. 5A); the study was terminated at week 5 because the tumors became necrotic. In the group of mice which received daily injection of 10 µg of [Psi13-14, Leu14]BN, the xenograft growth was slowed significantly by 72% at week 2 and by approximately 50% during weeks 3, 4, or 5 (Fig. 5A). These data indicate that [Psi13-14, Leu14]BN inhibits the growth of SCLC in vivo. In contrast, (APTTL)SP, which is a BN/GRP receptor antagonist approximately 30-fold weaker than [Psi13-14, Leu14]BN, only weakly inhibited tumor formation (Fig. 5A). In the second study, [Psi13-14, Leu14]BN significantly inhibited xenograft formation, whereas BN1-12, which is inactive, did not (Fig. 5B). Also, the BN/GRP receptor antagonist [Pyr6, Phe13, Psi13-14, Leu14]BN6-14 weakly inhibited NCI-H69 xenograft formation (Table 4); similar data were obtained for [o-Nal6, Psi13-14, Phe14]BN6-14. In the animals treated with [Psi13-14, Leu14]BN, tumors were excised and the tumor weight, DNA, and protein content were determined. Table 5 shows that tumors treated with [Psi13-14, Leu14]BN had significantly less weight and protein than did controls. Also, the DNA content was reduced by approximately 30% at the termination of the study.

**DISCUSSION**

Previously, we demonstrated that some lung cancer cells such as NCI-H520 and NCI-H727 have epidermal growth factor receptors and utilize TGFα but not BN/GRP as autocrine growth factors (28). In contrast, SCLC cell lines such as NCI-H69, H345, and N592 as well as the lung carcinoid NCI-H720 have BN/GRP receptors and utilize BN/GRP but not TGFα as autocrine growth factors (28). Here the ability of synthetic peptides to inhibit lung cancer cell line BN/GRP receptor binding, second messenger production, and growth was investigated.

Previously, we demonstrated that the carboxyl but not the amino terminal of BN or GRP was essential for high affinity binding SCLC cell line NCI-H345 or rat brain membranes (12, 29). To further improve antagonists of the BN/GRP receptor, [Psi13-14, Leu14]BN fragments which lack the NH₂ terminal of BN were synthesized and their potency was evaluated in receptor-binding assays. [D-Nal6, Psi13-14, Phe14]BN6-14 was the most potent analogue tested with an IC₅₀ value of 5 nM. [D-Nal6, Psi13-14, Leu14]BN6-14, [D-Phe6, Psi13-14, Leu14]BN6-14 and [Pyr6, Phe13, Psi13-14, Leu14]BN had IC₅₀ values of 15, 25, and 50 nM, respectively. Therefore, [D-Nal6, Psi13-14, Phe14]BN6-14 is approximately 1 and 2 orders of magnitude more potent than is [Psi13-14, Leu14]BN or (APTTL)SP. For reduced peptide bond analogues of BN, a hydrophobic amino acid analogue such as Nal is preferred at position 6 instead of Asn, and a hydrophobic amino acid such as Phe is preferred at position 14 for high affinity antagonist binding to SCLC BN/GRP receptors. Similarly, for guinea pig pancreatic acini and Swiss 3T3 cells, [D-Nal6, Psi13-14, Phe14]BN6-14, [D-Phe6, Psi13-14, Leu14]BN6-14, and [D-Nal6, Psi13-14, Leu14]BN6-14 were 2- to 10-fold more potent than was [Psi13-14, Leu14]BN (30).

The ability of the antagonists to inhibit BN-induced second messenger production was investigated by using cell line NCI-H345. Previously we found that BN elevated cytosolic Ca²⁺ levels in SCLC cells, possibly as a result of increased inositol-1,4,5-trisphosphate production (14, 15). [D-Nal6, Psi13-14, Phe14]BN6-14 had no effect on the cytosolic Ca²⁺ levels by itself but inhibited in a dose-dependent manner the increase in cytosolic Ca²⁺ caused by BN. Because the dose of BN was 10 nM, one micromolar [D-Nal6, Psi13-14, Phe14]BN6-14 was needed to totally abolish the increase in cytosolic Ca²⁺ caused by 10 nM BN. The BN receptor antagonist was reversible in that the inhibition caused by 1 µM [D-Nal6, Psi13-14, Phe14]BN was reversed by 1000 nM BN. Similar data were obtained for BN/GRP. [Psi13-14, Leu14]BN6-14, [Psi13-14, Leu14]BN6-14, and [Pyr6, Psi13-14, Phe14]BN6-14. Because the increase in cytosolic Ca²⁺ caused by 10 nM BN was totally blocked by 10 µM (APTTL)SP (21) or 3 µM [Psi13-14, Leu14]BN (22), reduced peptide bond analogues such as [D-Nal6, Psi13-14, Phe14]BN6-14 are approximately an order of magnitude more potent than is [Psi13-14, Leu14]BN or [D-Arg1, D-Pro2, D-Trp7, Leu11] substance P at inhibiting second messenger production.

The ability of the reduced peptide bond analogues of BN to inhibit SCLC growth in vitro was investigated. In a clonogenic assay, 1 µM [Psi13-14, Leu14]BN decreased the number of colonies to 24 and 39% of control, respectively, using cell lines NCI-H345 and NCI-N592. Because over one-half of the clonal growth was inhibited by the BN receptor antagonist, it would suggest that BN/GRP is an important autocrine growth factor for SCLC. The clonal growth may not be totally inhibited by BN/GRP receptor antagonists because SCLC cells may use other growth factors such as insulin-like growth factor 1 (31) or transferrin (32). Also, BN (10 nM) stimulated colony formation in both cell lines and in the presence of BN, 50 µM [Psi13-14, Leu14]BN decreased the number of colonies to 24 and 39% of control, respectively, using cell lines NCI-H345 and NCI-N592, respectively. Because 1 µM [Psi13-14, Leu14]BN greatly decreased the number of colonies in the presence or absence of 10 nM BN to approximately 30%, [Psi13-14, Leu14]BN decreased the growth of SCLC due to endogenous or exogenous BN/GRP in a dose-dependent manner. Similar data were obtained for [D-Nal6, Psi13-14, Phe14]BN6-14, [D-Nal6, Psi13-14, Leu14]BN6-14, and [D-Phe6, Psi13-14, Leu14]BN6-14. In contrast, [Psi13-14, Leu14]BN did not alter the growth of NCI-H520 (squamous cell carcinoma) and H727 (bronchial carcinoid) which use TGFα and not BN/GRP as autocrine growth factors (28). Also, a peptide of similar size and charge to [Psi13-14, Leu14]BN, BN1-12 did not bind to BN/GRP receptors on NCI-H345 with high affinity and did not alter the cyclosic Ca²⁺ or alter the clonal growth.

The ability of [Psi13-14, Leu14]BN to inhibit xenograft formation...
tion in nude mice was determined. Injection s.c. of 10 μg of [Ψ13-14, Leu14]BN inhibited NCI-N592 tumor volume by 72% in week 2 and by approximately 50% in weeks 3–5. The effects of [Ψ13-14, Leu14]BN were dose dependent in that injection of 0.1 μg of [Ψ13-14, Leu14]BN. As controls, injection of 10 μg of (Ψ13-14, Leu14), which is less potent than is [Ψ13-14, Leu14]BN, or BN-18, which is inactive, did not significantly alter NCI-N592 xenograft formation (Fig. 5). Also, 50 μg of [Ψ13-14, Leu14]BN and [Ψ13-14, Leu14]BN-18 daily significantly inhibited NCI-H69 xenograft formation by approximately 20% (Table 4). These data suggest that [Ψ13-14, Leu14]BN is less potent than is [Ψ13-14, Leu14]BN in vivo. This is surprising as in receptor binding studies [Ψ13-14, Leu14]BN was approximately as potent a BN/GRP receptor antagonist as was [Ψ13-14, Leu14]BN. It is possible, however, that [Ψ13-14, Leu14]BN-18 is degraded by endogenous proteases more rapidly than is [Ψ13-14, Leu14]BN. Previously, we found that NCI-N592 xenograft formation was inhibited when monoclonal antibody 2A11 (100 μg, i.p.) was injected 3 times weekly (18). A particular advantage of [Ψ13-14, Leu14]BN relative to 2A11, however, is that due to its small size, the synthetic peptide may have better tumor penetration relative to the mouse monoclonal antibody and will not be neutralized by host immune mechanisms.

In summary, reduced peptide bond analogues of BN function as SCLC BN/GRP receptor antagonists. They inhibit binding to SCLC BN/GRP receptors and BN-induced second messenger production. Also, [Ψ13-14, Leu14]BN inhibits SCLC growth using a clonogenic assay in vitro and xenograft formation in nude mice in vivo. It remains to be determined if these BN/GRP receptor antagonists will function as useful therapeutic agents in the treatment of SCLC.

REFERENCES

[Psí^{13,14}] Bombesin Analogues Inhibit Growth of Small Cell Lung Cancer \textit{in Vitro} and \textit{in Vivo}

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